Saccharomyces cerevisiae α-factor prevents formation of glycoproteins in *a* cells

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Received 3 June 1983

α-Factor inhibits incorporation of [^{14}C]glucosamine into water-soluble and into SDS-extractable glycoproteins to about 90%. The incorporation into chitin is not affected and the same is true for [^{14}C]phenylalanine incorporation into protein. The inhibition of glycoprotein synthesis is specific for *a* cells.

Cell cycle *α-Factor* *Glycoprotein* *G1 arrest* *Yeast*

1. INTRODUCTION

Saccharomyces cerevisiae cells show a first cycle arrest in G1 when *N*-glycosylation of proteins is inhibited by tunicamycin [1]. The same was observed with a temperature-sensitive mutant defective in the synthesis of the glycosylation precursor Dol-PP-$GlcNAc_2Man_9Glc_3$ [2]; at the non-permissive temperature all cells completed their cell division cycle until G1 [3]. Therefore, yeast may require at least one *N*-glycosylated protein for the G1/S phase transition. Further, when synchronized with α-factor, haploid cells of mating type *a* failed to start S phase, when, after removal of α-factor, *N*-glycosylation was prevented [1,3]. This indicated that in the presence of α-factor, cells were not able to accumulate the postulated glycoprotein(s). To test this conclusion, *a* cells were incubated with [^{14}C]glucosamine in the presence and absence of α-factor. The results obtained show that α-factor strongly inhibits the formation of glycoproteins.

2. MATERIALS AND METHODS

α-Factor was obtained from Bachem (Basel). [1-^{14}C]Glucosamine, [^{14}C]phenylalanine and [2-^{3}H]mannose were from Amersham-Buchler (Braunschweig) and [^{14}C]glucose from NEN (Dreieich).

The haploid strains of opposite mating type, *S. cerevisiae* X 2180-1*a* and X 2180-1α, as well as the isogenic diploid strain X 2180-a/α were used here. Cells were grown overnight in defined medium [4] containing 2% (w/v) sucrose, then diluted to A_{578} of 0.6 in fresh defined medium containing 0.6% (w/v) sucrose, and grown for further 4 h. Cells from this exponentially growing culture were harvested and diluted to A_{578} of 0.6 in fresh prewarmed defined medium containing 0.6% sucrose. All incubations were at 30°C.

α-Factor (final conc. 10 μg/ml) was added at this stage, and incubation continued for 30 min. Control cultures, without α-factor, were incubated in parallel, after which the incorporation of [1-^{14}C]glucosamine, [^{14}C]phenylalanine, [^{14}C]glucose or [2-^{3}H]mannose into trichloroacetic acid-insoluble material was measured.

Radiolabelled cells were harvested by centrifugation, washed once with ice-cold buffer (10 mM Tris–acetic acid (pH 7.6) containing 0.2 M NaCl, 1.0 mM $CaCl_2$, 1.0 mM $MnCl_2$, 0.5 mM $Mg(CH_3COO)_2$ and 1.0 mM phenylmethylsulphonyl fluoride) and resuspended in 0.5 ml buffer. Mechanical breakage of cells was affected by

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00145793/83/$3.00

vortex mixing with glass beads (Braun, Melsungen; 0.45–0.50 mm diam.) for a total of 5 min with intermittent cooling. The broken cell slurry was removed and the beads washed 3 times with 0.5 ml buffer. The pooled cell slurry and washings were centrifuged at 42000 × *g* for 45 min at 2°C, and the supernatant removed. The 42000 × *g* pellet was washed once with 1 ml buffer and centrifuged as before. The supernatants (water-soluble fractions) were pooled, and their radioactivity measured. No free radiolabelled glucosamine or mannose was detected in the water-soluble fractions from cells incubated with these respective sugars. The pellet was resuspended in 0.5 ml 1.5% (w/v) sodium dodecyl sulphate (SDS), and heated at 98°C for 3 min, then centrifuged at 30000 × *g* for 20 min at room temperature. This procedure was repeated once with the pellet, and the radioactivity of the pooled supernatants (SDS-soluble fraction) was measured. No further radioactivity was extracted with higher concentrations of SDS.

The SDS-insoluble pellet was resuspended in water, and its radioactivity measured. This pellet was further extracted by heating at 98°C with 0.5 ml 1.0 M HCl for 15 min, after which insoluble material was sedimented by centrifugation. The extraction with HCl was repeated once and the residue then extracted twice with 1.0 M NaOH under the same conditions. The radioactivity in the acid and alkali extract, and in the insoluble material, was measured.

SDS–polyacrylamide gel electrophoresis was done as in [5].

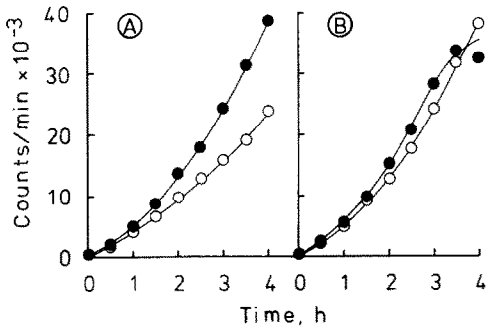

Fig.1. Effect of α-factor on (A) glucosamine and (B) phenylalanine incorporation. *Saccharomyces cerevisiae* X 2180-1a was grown as in section 2. α-Factor (final conc. 10 μg/ml) was added to one sample (10 ml), and incubation of this culture, and of a control, was continued for 30 min, whereupon 5 μCi [1-^{14}C]glucosamine (54 mCi/mmol) (A), or 5 μCi [^{14}C]phenylalanine (2–5 mCi/mmol) (B) was added at time 0. Portions (0.25 ml) were analysed for radioactive trichloroacetic acid-precipitable material: (●) control incubations; (○) incubations with α-factor.

3. RESULTS

Whereas α-factor shows almost no effect on protein synthesis, in accordance with [6], it significantly decreased the incorporation of [^{14}C]glucosamine into trichloroacetic acid-precipitable cell material (fig.1). The inhibition varied from 30–50%; however, it was considerably more pronounced in certain fractions after the radiolabelled cells were fractionated according to table 1. The incorporation of [^{14}C]glucosamine in-

Table 1

Effect of α-factor on the distribution of radiolabelled glucosamine and phenylalanine among cell fractions of *Saccharomyces cerevisiae* X 2180-1*a*

Radiolabelled precursor	Incubation	Fraction (total cpm × 10^{-3})		
		Water-soluble	SDS-soluble	Pellet
[1-^{14}C]Glucosamine	Control	166.6	116.9	619.2
	+ α-factor	22.2	15.2	449.8
[^{14}C]Phenylalanine	Control	623.3	396.3	50.2
	+ α-factor	696.8	441.1	74.7

The cultures radiolabelled for 4 h with [1-^{14}C]glucosamine or [^{14}C]phenylalanine in the experiment of fig.1 were harvested and fractionated as detailed in section 2. The total radioactivity in the water-soluble, SDS-soluble and SDS-insoluble (= pellet) fractions was determined

Table 2

Further fractionation of the SDS-insoluble, [^{14}C]glucosamine-labelled pellet

Incubation	Fraction (total cpm × 10^{-3})	
	Acid + alkali soluble	Chitin
Control	391.7	431.3
+ α-factor	159.6	485.0

[1-^{14}C]Glucosamine-labelled, SDS-insoluble pellets were obtained on fractionation of *S. cerevisiae* X 2180-1*a* cells radiolabelled in the absence and presence of α-factor using the method in table 1, and successively extracted at 98°C with 1.0 M HCl and 1.0 M NaOH, as detailed in section 2. Total soluble and insoluble (= mainly chitin) radioactivity was determined

to water-soluble and into SDS-extractable glycoproteins was inhibited by up to 90%, whereas the effect on non-extractable glycoproteins plus chitin was small. When the non-solubilizable polymers were treated with hot acid and base to remove all material but chitin [7] there was no difference in the radioactivity of the residual chitin or at best even a slight increase (table 2). That the remaining insoluble material indeed is chitin was supported by the observation that 80% of this radioactivity was degraded by chitinase (not shown). The results of table 1 also show that α-factor does not affect the incorporation of [^{14}C]phenylalanine into any of the various fractions.

To show whether α-factor prevents synthesis either of certain specific glycoproteins, or of all glycoproteins, the water-soluble and SDS-soluble fractions were separated by gel electrophoresis. The water soluble fraction separates into 6–7 radioactive bands, the SDS extract into about 13 (fig.2). All these radioactive bands are drastically reduced in intensity in the extracts from α-factor treated cells, although 2–3-fold larger aliquots were put onto the gel. Again, no difference is observed in the extracts labelled with [^{14}C]phenylalanine (fig.2, columns 3,4 and 7,8).

Glycoproteins in *S. cerevisiae* are exclusively mannoproteins [8,9]. If α-factor indeed stops the synthesis of all glycoproteins it should also affect the incorporation of [2-^{3}H]mannose into polymers. This is the case (table 3). The effect is less pronounced than that with glucosamine, which is not unexpected; however, since halt in glycoprotein synthesis would not immediately stop the considerable extension of prefabricated core chains [8,9]. A control with [^{14}C]glucose shows that its incorporation into trichloroacetic acid-insoluble material is not significantly inhibited, except a slight effect on the incorporation into insoluble material. This could be due to a conversion of some glucose into mannose.

Table 4 clearly demonstrates that glucosamine incorporation into trichloroacetic acid-insoluble material is only affected in *a* cells, not in α cells and not in diploid a/α cells. Thus, α-factor inhibits glycoprotein synthesis specifically in *a* cells.

Table 3

Effect of α-factor on the distribution of radiolabelled glucose and mannose among cell fractions of *S. cerevisiae* X 2180-1a

Radiolabelled precursor	Incubation	Fraction (total cpm × 10^{-3})		
		Water-soluble	SDS-soluble	Pellet
[^{14}C]Glucose	Control	267.9	209.7	235.4
	+ α-factor	279.8	199.9	186.7
[2-^{3}H]Mannose	Control	513.5	945.0	2993.6
	+ α-factor	377.0	697.6	1616.5

Incubation conditions were as for fig.1 except that radiolabelling was with either 5 μCi [^{14}C]glucose (348 mCi/mmol) or 50 μCi [2-^{3}H]mannose (13.4 Ci/mmol). After 4 h incubation, cells were harvested and fractionated as detailed in section 2. The total radioactivity in water soluble, SDS-soluble and SDS-insoluble fractions was determined

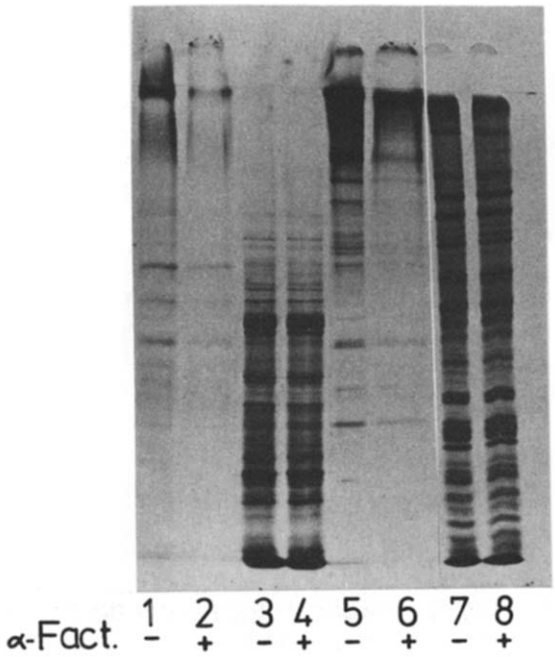

Fig.2. Effect of α-factor (10 μg/ml) on glucosamine and phenylalanine incorporation into individual proteins and glycoproteins (fluorogram). X 2180-1a cells were radiolabelled with [1-^{14}C]glucosamine (spec. act. 54 mCi/mmol), lanes 1,2 and 5,6, and with [^{14}C]phenylalanine (spec. act. 3 mCi/mmol), lanes 3,4 and 7,8. The incubation was carried out in a total volume of 20 ml (A_{578} 0.88) for 105 min in the presence of 40 μCi glucosamine and 20 μCi phenylalanine, respectively. Cells were harvested and fractionated as detailed in section 2. Lanes: (1–4) water-soluble fraction (25, 60, 7 and 7 μl); (5–8) SDS-soluble fraction (30, 80, 7 and 7 μl).

Table 4

α-Factor only inhibits incorporation of [1-^{14}C]glucosamine by *S. cerevisiae a* cells

Incubation	Incorporation of cpm (× 10^{-3}) into trichloroacetic acid-precipitable material		
	X2180-1a	X2180-1α	X2180-a/α
Control	37.0	17.9	45.2
+ α-Factor	22.5	17.9	44.0

S. cerevisiae strains X 2180-1a, X 2180-1α and X 2180-a/α were grown and radiolabelled with [1-^{14}C]glucosamine in the absence and presence of α-factor (10 μg/ml) as in fig.1. After 4 h, aliquots (0.25 ml) were analysed for radioactive trichloroacetic acid-precipitable material

4. DISCUSSION

α-Factor, a peptide pheromone of known structure [10], is released by α cells into the medium and prepares cells of the opposite mating type (*a* cells) for conjugation. These cells are arrested in G1 [11], show pear-shaped growth called 'shmoo' [12] and, due to changes in their cell surface [13], *a* cells agglutinate with α cells as a prelude to mating [14]. α-Factor induces all these changes in *a* cells after being bound to a receptor on the cell surface [15], possibly without having to enter the cell. Little is known, however, about intracellular changes brought about by α-factor, which lead to the phenomena described. Here a quite dramatic intracellular change, and a specific one for *a* cells, is reported. Whether the inhibition of glycoprotein synthesis by α-factor causes G1 arrest of *a* cells, or whether it is merely a consequence of the fact that in the presence of α-factor and with time, increasingly more cells become G1 cells, which might be perturbed in glycoprotein synthesis, cannot be concluded yet. However, the former possibility seems more likely, since the inhibition observed stays constant (fig.1), although the unbudded cells (G1) increase from time 0 to 2 h from 50–95% [1,3]. In addition, when *N*-glycosylation of proteins is prevented by tunicamycin or by non-permissive temperatures for a corresponding ts-mutant [1,3] this actually causes G1 arrest.

α-Factor does not inhibit the incorporation of glucose into polymeric material. Since glucosamine as well as mannose use the same transport system as glucose, the α-factor effect cannot be one on transport of the labelled precursors. In addition, glucosamine is a precursor not only in protein glycosylation but also in chitin synthesis. Nevertheless, its incorporation into chitin is not at all inhibited by α-factor (table 2), whereas the inhibition of incorporation into extractable glycoproteins amounts to 90% (table 1). This inhibition is in accordance with the observation that α-factor treated cells contain less mannan [13].

Although α-factor behaves in many ways like a 'physiological tunicamycin', it remains to be resolved whether: (i) it indeed somehow prevents protein glycosylation; or (ii) it prevents synthesis of those proteins which are destined for 'secretion' into the lumen of the endoplasmic reticulum; e.g., by interference at the level of the signal recognition particle [16].

ACKNOWLEDGEMENTS

Thanks are due to Dr Norbert Sauer for helpful advice. This work has been supported by the Deutsche Forschungsgemeinschaft (SFB 43).

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